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HETEROTHERMY, TORPOR, RESPIRATORY GAS EXCHANGE, WATER BALANCE AND THE EFFECT OF FEEDING IN GOULD'S LONG-EARED BAT *NYCTOPHILUS GOULDI*

STEVE MORRIS, ALISON L. CURTIN AND MICHAEL B. THOMPSON
School of Biological Sciences (A08), University of Sydney, Sydney, NSW 2006, Australia

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Summary

The effects of temperature and nutritional status on the metabolic rate of *Nyctophilus gouldi* were examined. Bats fed marked meals first defecated approximately 1.34 h after feeding and were calculated to have a mean retention time of 5.38±0.57 h but to be truly post-absorptive after 9 h. Over the temperature range 1–35 °C, the metabolic rate ($\dot{V}_{O_2}$) and body temperature (T_b) of fasted bats were extremely labile. Below 30 °C, the bats all entered torpor and between 10 and 15 °C showed a mean 84 % reduction over the maximal $\dot{V}_{O_2}$. Body temperature was also minimal over this range (T_b=12.5 °C, T_a=10–15 °C). Both total and dry thermal conductance increased in a curvilinear manner with temperature, total conductance from 3.38±0.65 J g^{-1} h^{-1} °C^{-1} at 1 °C to 24.25±1.99 J g^{-1} h^{-1} °C^{-1} at 35 °C (mean ± S.E.M.), while the rate of evaporative water loss increased with T_a by a maximum of 10-fold from 0.21 mg g^{-1} h^{-1} at 5 °C to 2.69 mg g^{-1} h^{-1} at 35 °C. Between 10 and 25 °C, intermittent respiration characterised by episodic bouts of breathing/gas exchange and periods of apnoea with no measurable $\dot{V}_{O_2}$ occurred. Although the duration of apnoea decreased when temperature was increased, the volume of oxygen taken up in each episode did not change. Mean respiratory exchange ratio (RER) was low (0.64–0.77) in post-absorptive bats, typical of fat utilisation, but during torpor ranged from near 0 to near 2, indicating discontinuous and disproportional gas exchange. Feeding produced a condition of relatively sustained homeothermy and high RER in the bats which persisted for 9 h, after which the *N. gouldi* became torpid. Immediately after feeding, the $\dot{V}_{O_2}$ of the bats increased fivefold above the post-absorptive level, while the $\dot{V}_{CO_2}$ increased by more than eightfold. Similarly, body temperature also increased, declining to torpid values after 9 h. The RER in immediately post-feeding bats was near 1.0 but subsequently declined to near 0.7, indicating a switch from carbohydrate to fat utilisation. Therefore, the *N. gouldi* were heterothermic, exhibited a highly labile metabolic rate, and rates of heat and water loss, and a T_b which were influenced both by ambient temperature and by nutritional status.

Introduction

At low air temperatures, small endotherms may save large amounts of energy by abandoning constant body temperature (homeothermy) and becoming torpid (Lyman

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et al. 1982). Torpor in endotherms is characterised by a sizeable reduction in metabolic rate accompanied by a depression of body temperature (Lyman *et al.* 1982). Small mammals such as bats have high mass-specific metabolic rates in normothermy, but lack large fat stores from which energy can be obtained. As a result, their internal energy reservoirs deplete rapidly and, consequently, energy must be supplied by frequent feeding. For example, Speakman and Racey (1987) calculated that fat stores in the long-eared bat *P. auritus* would last 1 or 2 days only.

In most bats, feeding necessarily requires flight, which presents a further energy demand. The addition of flight membranes increases the surface area of the body (Twente, 1955) in a complex fashion since the additional area varies according to the relative duration of flight; the wings are folded during roosting. These flight membranes potentially provide bats with a total surface area approximately six times greater than that of wingless mammals of the same body mass (Phillips, 1984). The wings, tails and often ears of bats are large, naked and highly vascularised (Wells, 1971) and at least partly exposed at all times. Consequently, rates of thermal conductance and water loss are high (Bradley and Deavers, 1980). Further, the often nearly constant food supply (and feeding) required by insectivorous bats is substantially reduced in winter, which coincides with a period of potentially increased energy demands (Lyman *et al.* 1982). This energetic dilemma has been solved by heterothermy.

Although there is a history of research on heterothermy in bats, mostly on species from the northern hemisphere (e.g. Kurta *et al.* 1987; Kurta and Kunz, 1988; Audet and Fenton, 1988), there has recently been detailed consideration of the breathing patterns and the effect of nutritional status on torpor in bats (see below). Apnoea during torpor or hibernation, termed intermittent respiration or Cheyne–Stokes breathing, has been observed in a number of small mammals and may be associated with reduced metabolic rate (Milsom, 1991). Appreciation of the potential importance of episodic breathing and discontinuous gas exchange to energy balance in bats is reflected in a number of recent studies (e.g. Chappell and Roverud, 1990; Thomas *et al.* 1990; Hays *et al.* 1991; Szewczak and Jackson, 1992*a*,*b*; Thomas and Cloutier, 1992). These important contemporary studies have concentrated largely on the mode, mechanisms and route of oxygen uptake. The reported $\dot{V}_{O_2}$ values are diverse and apparently species-dependent, stimulating debate as to whether the glottis of apnoeic bats prevents the diffusive entry of oxygen (see Thomas and Cloutier, 1992; Hays *et al.* 1991). There are, however, other important issues in addition to oxygen uptake. There are almost no contemporary reports of CO_2 excretion rates (see however, Chappell and Roverud, 1990) and none for apnoeic/torpid microchiropterans. Thus, most available estimates of $\dot{V}_{CO_2}$ and respiratory exchange ratio (RER) are extrapolations and remain speculative. Thomas *et al.* (1990) were required to assume a respiratory quotient (RQ) of 0.7 to estimate $\dot{V}_{CO_2}$, while Szewczak and Jackson (1992*b*) point out that 'following the respiratory quotient through a ventilatory bout would be informative'. Metabolic rate and ventilation volume vary proportionally at fixed temperature and, since ventilation is 'CO_2 driven', arterial P_{CO_2} tends to be constant throughout the metabolic scope (Dejours, 1981). In heterothermic mammals, decreased temperature alters a number of physical constants, implying that the ventilatory 'set point' for CO_2 must be adjusted (Szewczak and Jackson, 1992*a*,*b*). These

workers (Szewczak and Jackson, 1992*a*) also provide strong evidence that CO_2 storage occurs in apnoeic bats and that it influences breathing patterns. Furthermore, considering that torpor, feeding and the costs of feeding are factors in metabolic efficiency, i.e. the cost of energy acquisition and production, the paucity of data relating gas exchange to acid–base balance severely limits our understanding of the importance of torpor in modifying respiratory gas exchange and energy utilisation/conservation.

The underlying assumption of investigations of basal metabolism in bats (e.g. McNab, 1969; Phillips, 1984; Kurta and Kunz, 1988) is that the animals are post-absorptive, i.e. that all food has been digested. Previous studies have not always justified this assumption, and determinations for each species are required. Food processing, including mechanical processing, transport, storage or utilisation of products, will result in overestimates of basal metabolic rate (Kleiber, 1961). Passage of digesta through the gut is best described by mean retention time (MRT) (Warner, 1981). Warner (1981) concluded that the shortest MRT (1–6 h) occurs in small mammals, which might be expected to include microchiropteran bats. Flying foxes also appear to be in this range when fed fruit (2–4.5 h; Richardson *et al.* 1987), as do smaller pollen-feeding megabats such as *Syconycteris australis* (approximately 2.5 h; Law, 1991). It can thus be expected that small insectivorous bats should have a short MRT and rapidly become post-absorptive. This must be verified, however, in any study of hypometabolism.

The current study investigated the effect of temperature and nutritional status on the metabolic rate of the southern, temperate-zone bat, *Nyctophilus gouldi* (Vespertilionidae). The genus *Nyctophilus* contains six species considered endemic to Australia (Hall, 1981). The genus is distinguished by very large ears and a small simple nose-leaf. *N. gouldi* are common in south-eastern and south-western Australia in dry sclerophyll forest and savannah woodland habitats (Richards, 1983). *N. gouldi* are tree-dwelling bats and are relatively gregarious by nature, occurring under bark and in tree hollows in colonies of up to 25 bats. The species feeds exclusively on insects captured in flight or gleaned from vegetation. In the southern part of their range, *N. gouldi* hibernate in the winter when prey are scarce and during other seasons may enter daily torpor depending on environmental conditions (Richards, 1983). The present study examines the propensity for torpor, the association of intermittent gas exchange with torpor, metabolic rate and temperature and also the effect of feeding on these relationships.

Materials and methods

Nyctophilus gouldi (Tomes) (N=25) were collected under permit in Olney State Forest, NSW, between March and August 1991 (Autumn–Winter) using harp traps. Numbers and usage were limited by permit: National Parks and Wildlife Service (NSW) licence B816; Forestry Commission of NSW, special purposes permit 03915. The bats were taken to the University of Sydney and held individually in cages (0.37 m×0.24 m×0.24 m high) with a 12 h:12 h L:D photoperiod at 22±2 ˚C. The bats were flown twice weekly in a larger enclosure (2 m×4 m×3 m) until they voluntarily roosted for at least 5 min, were fed mealworms (*Tenebrio* sp.) nightly and fresh water was supplied *ad libitum*. The bats were acclimated to these conditions for at least a week prior to preliminary experimentation, to

stabilise body mass (range 8.0–10.5 g). The bats weighed the same when released at the end of the experiment as at the end of week 1.

Determination of food passage rate

Rate of food passage is usually measured by labelling the food with an indigestible marker. Phenol Red can be used as such a marker (I. D. Hume, personal communication). However, *N. gouldi* selectively fed on 'unmarked' mealworms in preference to those injected with Phenol Red. Glass beads and pollen have also been successfully employed (Kotb and Luckey, 1972). Pollen is both small (20–75 μm diameter) and hard and was therefore tested for bats. Mealworms were rolled in a pollen mix of native species, *Banksia*, *Grevillea* and Myrtaceae, and fed to two bats. Faeces were collected, crushed and viewed under a microscope. Pollen appeared in sufficient amounts (zero to several hundred grains) to detect differences between defecations reliably. Lactophenol Blue stains only intact pollen grains; empty, i.e. digested, pollen does not take up the stain. This method showed that *N. gouldi* did not digest pollen.

Five *N. gouldi* were isolated in separate enclosures and fed mealworms rolled in pollen until satiated. For the first 2 h after feeding, the enclosures were checked at 5 min intervals to determine the time of first defecation (t_0). During the subsequent 10 h, the enclosures were checked every 30 min, then at 15, 24 and 25 h post-feeding. All faeces were collected and the defecation time noted; the bats received a meal of unmarked mealworms at 24 h as part of their normal feeding regime.

Each defecation was homogenised in 400 μl of water and three 80 μl subsamples were removed. The number of pollen grains in nine fixed (non-overlapping) fields of view (3.1 mm^2) were counted. It was not possible to determine exactly the amount of ingested pollen and therefore a cumulative count of pollen in the faeces was made.

Times of mean retention of pollen within the gut were calculated using the equation:

$$\mathrm{MRT} = \sum_{i=1}^{n} m_i t_i / \sum_{i=1}^{n} m_i ,$$

where m_i is the amount of pollen excreted at the ith defecation, at time t_i after feeding of the marked meal and n is the total number of defecations (Warner, 1981).

Gas exchange

With exception of the effect of feeding determinations (see below), rates of oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide excretion ($\dot{V}_{CO_2}$) were determined for post-absorptive (24 h fasted) bats (N=17) during their resting phase (daylight) using flow-through respirometry. Each bat was exposed to all temperatures in a random order. Preliminary experiments showed that the $\dot{V}_{O_2}$ during an ascending temperature series was the same at each temperature as during a descending temperature series. Nonetheless, measurements at randomly selected temperatures were repeated for the bats to check any deviation in the response. The respirometer was contained within an internally illuminated (resting phase) temperature-controlled cabinet to provide constant temperatures over the range 1–35 °C and recordings were made at 5 °C intervals. Humidified air (63 % relative humidity) was drawn through the system by a pump at 100 ml min^{-1}. The optimum flow rate was established experimentally at 35 °C as a compromise between chamber wash-out time and

hypoxia/hypercapnia. The maximum gas exchange deviation was monitored on the oxygen line (mean $\dot{V}_{O_2} > \dot{V}_{CO_2}$) such that percentage O_2 never declined by more than 0.6 %. While the CO_2 level in the chamber was usually less than 0.5 %, peak values near 0.8 % were occasionally recorded. The humidity was maintained in the respiration chambers using NaOH solutions specified in the tables of Klekowski (1975), which also removed CO_2 prior to passage through the chambers (chamber volume 64.5 ml), at each of the temperatures employed. The humidifier was replaced for each run (approximately 2.5 h) and humidity was occasionally verified using a meter housed in a 500 ml chamber, in place of the respirometer. Modified and calibrated Ametek R2 flow controllers with additional float flow controllers and meters were as described by Speakman *et al.* (1991). Each configuration was calibrated repeatedly, using a variety of bubble calibration tubes, for at least 10 flow rates between 10 and 400 ml min^{-1}. Regression of the exponential calibration plots always gave $r^2 > 0.94$. Excurrent air from the chambers was passed through a 'Drierite' filter (approximately 10 ml) to absorb water, through a carbon dioxide analyzer (Ametek model CD-3A) and then through Carbosorb (approximately 10 ml) to absorb the remaining CO_2 prior to passage through a small Drierite 'guard' and an oxygen analyzer (Ametek model S-3A). The O_2 analyzer was used in differential mode ($F_I - F_E$). The reference air supply was passed through the same humidifying solution, through a dummy respirometer balanced to the animal chamber by a water manometer and through a pre-weighed 10 ml Drierite filter before entering the O_2 analyzer. The reference Drierite filter allowed determination of the water originating from the humidifier rather than the animal. On occasions when bats urinated or, more rarely, defecated in the chambers, the run was aborted and the data were discarded. The O_2 analyzer was calibrated with the same room air fed to the respirometer, after it had been dried. The CO_2 analyzer was calibrated using 0.5 % and 7 % CO_2/N_2 analyzed and certified gas mixtures, and the calibration was checked with a 3.2 % CO_2/N_2 gas mixture. Occasionally, a double check was made using a M10a/f Wösthoff gas-mixing pump. Output was recorded using Sable System's Datacan V data acquisition system at a rate of 0.05 s^{-1}, averaged and displayed for analysis at rates of 0.5–10 s^{-1} proportional to the length of the record. Recordings (30 min) of the steady-state condition were made at each temperature. However, it must be noted that the CD-3A CO_2 analyzer has a digitised output to both display and analog outputs such that resolution is usually set at 0.01 %. As a consequence, while it is possible to be confident about high $\dot{V}_{CO_2}$ readings (e.g. 0.5 %) and to accept the physiological significance of readings close to zero (i.e. no important difference between 0 and 0.01 %), the machine-imposed error can be significant in low $\dot{V}_{CO_2}$ readings.

$\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated using the Sable Systems software based on the equations of Withers (1977). $\dot{V}_{CO_2}$ was calculated as ml g^{-1} h^{-1} using the equation:

$$\dot{V}_{CO_2} = F(F_{ECO_2} - F_{ICO_2})$$

and $\dot{V}_{O_2}$ was calculated using the equation:

$$\dot{V}_{O_2} = F(F_{IO_2} - F_{EO_2})/(1 - F_{IO_2}),$$

where F is the STP-corrected flow rate, and F_E and F_I are excurrent and incurrent flow, respectively.

Using the Sable software, wash-out curve data can be obtained by introducing a N_2 bolus and storing the curve for each experimental set-up. Following the generated wash-out equations and procedure of Bartholomew *et al.* (1981), the data were transformed to instantaneous gas exchange values.

Temperature

Thermocouples in the air entering and leaving the chambers were used to determine the extent to which the chamber temperature deviated from that of the cabinet. The body temperature of the bats (T_b) was measured at the end of each recording within 30 s of their removal from the chamber using a lubricated copper–constantan thermocouple inserted 1 cm into the rectum. During the feeding measurements, continuous recordings were made of body surface temperature (T_{sur}) with thermocouples fixed between the shoulders of the animals.

Water loss and thermal conductance

Total evaporative water loss (TEWL) was routinely measured gravimetrically by weighing the bat and its chamber at the beginning and end of each $\dot{V}_{O_2}/\dot{V}_{CO_2}$ recording. The *N. gouldi* were resident in the respirometer for less than 2 h and, since they were post-absorptive (see MRT below), produced no faeces and only rarely was a measurement rejected because of urine production. Preliminary experiments using six bats at a variety of temperatures compared water loss determined directly from bat mass with that derived from a comparison of the mass change of the Drierite filters in the reference and experimental gas lines. Comparison of paired values (*t*-test) revealed no significant difference, simplifying the experimental approach. Calculations from measured $\dot{V}_{CO_2}$ for bats metabolising fat (RER=0.7) showed that the mass of O_2 fixed as metabolic water was very similar to the carbon mass lost as CO_2, the difference constituting approximately 2–3 % of the total water loss over the range 1–35 °C, which was also not significant. Total 'wet' thermal conductance (C_{TOTAL} in $J\,g^{-1}\,h^{-1}\,°C$), including evaporative heat loss, was calculated for RERs of 1.0 and 0.70, for carbohydrate and fat oxidation, respectively (McNab, 1980). Energy values were calculated from $\dot{V}_{O_2}$ ($ml\,g^{-1}\,h^{-1}$) using standard conversion factors of 21.31 and 19.8 $J\,ml^{-1}$ respectively (Gordon, 1977). This value was divided by the temperature differential between the bat and the chamber to provide a total conductance value as either mass-specific $J\,h^{-1}\,°C^{-1}$ ($J\,g^{-1}\,h^{-1}\,°C^{-1}$) or $W\,°C^{-1}$. Similarly 'dry' thermal conductance (C_{DRY}) can be derived as described by Chappell and Roverud (1990):

$$C_{DRY} = (MHP - EHL)/(T_b - T_a),$$

where MHP is metabolic heat production = $\dot{V}_{O_2}$ ($ml\,O_2\,g^{-1}\,h^{-1}$) × 19.83 ($J\,ml^{-1}\,O_2$), EHL is evaporative heat loss = TEWL ($g\,H_2O\,g^{-1}\,h^{-1}$) × 2404 ($J\,g^{-1}\,H_2O$).

Breathing patterns and episodic gas exchange

Patterns of breathing were determined for five *N. gouldi* over the temperature range 1–35 °C. Ventilation was initially monitored by continuous observation, but it soon became apparent that this was observable only when $\dot{V}_{O_2}$ was significantly above zero. Therefore, in later experiments, ventilation was confirmed whenever $\dot{V}_{O_2}$ was detectable.

When breathing was episodic with periods of apnoea, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were described as intermittent to distinguish them from stable or irregular gas exchange. For periods of intermittent breathing and gas exchange, the frequency of breathing episodes (min^{-1}), the integrated area of gas exchange during the breathing episodes (to provide volume of gas per episode in $ml\,g^{-1}$) and the length of apnoeic bouts (min) were measured.

The effects of feeding

$\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and body surface temperature (T_{sur}) of 12 *N. gouldi* were measured over 12 h at 25 °C. Six of these bats were fed mealworms equalling 15 % of their body mass prior to initiating measurements, while the remaining six were deprived of food. The differences between the two groups allowed the determination of the relative magnitude and duration of the digestive process, as well as the effects on body temperature, gas exchange and the propensity for torpor.

The data showing the effects of feeding were analyzed using two-factor analyses of variance (Systat 5.01). Homogeneity of variance was tested using Cochran's test (Winer, 1971). Multiple comparisons of means were performed using the SNK test. In the analysis of variance (ANOVA), time and treatment (fed *versus* unfed) were the factors. Time could be included as a factor by randomly selecting two bats from each treatment for each of the times, 2, 5 and 9 h after feeding. Analyses of repeated measures could have been employed for these data. However, such analysis relies on large sample sizes to 'dilute' biases imposed by repeated sampling of individuals. Sample sizes in this study were too small to justify this and the more robust method was used (Snedecor and Cochran, 1987). Otherwise, unless stated, *t*-tests have been used for the comparison of paired data and ANOVA for independent data sets, using a fiducial limit of P=0.05. All results are expressed as means ± standard errors.

Results

Food passage rate – time to post-aborption

Each bat defecated at irregular intervals between 7 and 12 times over the 25 h of observation. The first faeces, representing food transit time (t_0), were collected 1.34±0.04 h after the meal. These faecal pellets all contained some pollen marker, ranging from 3.2 to 21 % of the total. The peak defecation of the marker occurred 3.66 h post-feeding for four out of five bats (bat 5 peaked at 3 h) with a mean value for peak marker defecation of 3.53±0.15 h for all five bats. The amount of marker at the peak ranged from 34 to 54 % of the total. After the peak clearance, the pollen in the faeces rapidly decreased to 20 % and lower. During the period 10–25 h, very low (1–2 %) marker content was observed (Fig. 1).

The cumulative count of the marker in the faeces (Fig. 1) showed that a large percentage of the marker (70 %) was excreted within 5 h of feeding. The mean retention time for the marker (MRT) was calculated to be 5.38±0.57 h. This ranged from 4.14 to 6.68 h for the five bats.

Body temperature

The body temperature (T_b) of post-absorptive *N. gouldi* decreased when ambient

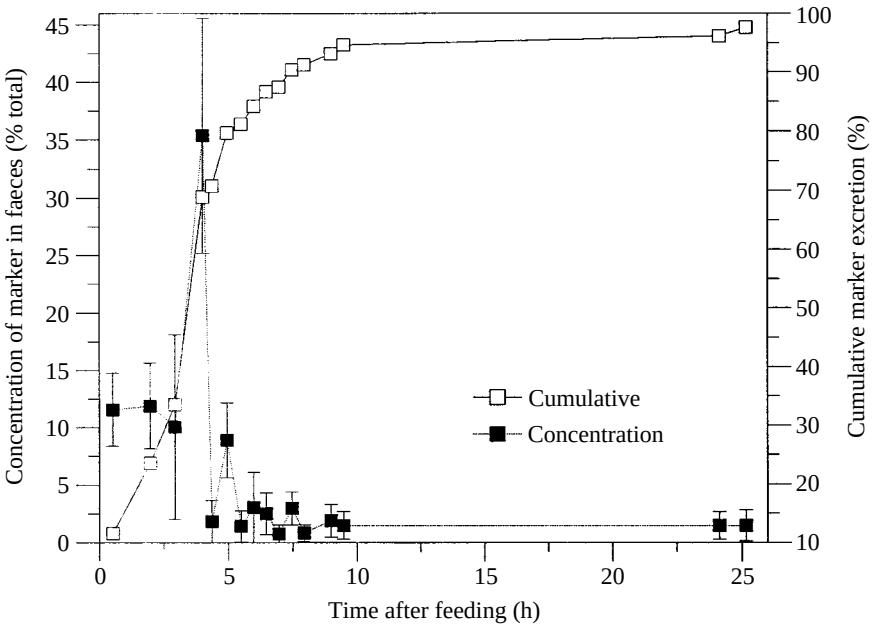

Fig. 1. The clearance from the digestive tract of pollen marker in the food of five *Nyctophilus gouldi*. Shown as concentration of marker (% total) in each defecation and as cumulative marker excretion. Values are mean ± S.E.M.

temperature (T_a) decreased, so that at T_a=35.3 °C, T_b was approximately 37.6 °C but T_b decreased to 9.9 °C at T_a=2.9 °C (Fig. 2). Thus, T_b did not vary linearly with T_a. The relationship between T_a and T_b can be described by an interpolative curve. However, examination of the metabolic rate/T_a curve (below) suggests two, or even three, phases in the $\Delta T_b/\Delta T_a$ curve; as T_a decreases to 15 °C, metabolic rate progressively decreases, but below 15 °C and down to 1 °C there is a progressive elevation of metabolic rate (see below).

The thermal strategy of *N. gouldi* thus appeared to be dichotomous and a test of hetergeneity of slopes (ANCOVA) was performed on the T_a/T_b data. The slopes of the regressions of T_b between 1 and 15 °C and between 15 and 35 °C were significantly different from each other ($F_{1,14}$=9.584). The slope of T_b between 1 and 15 °C did not therefore follow T_a, and between 15 and 35 °C, T_b statistically followed T_a.

Heat and water loss

Total evaporative water loss (TEWL) generally declined with a decrease in T_a (Fig. 3A). The minimum TEWL at 5 °C (0.21 mg $H_2O\,g^{-1}\,h^{-1}$) was only 8 % of the maximum rate (2.69 mg $H_2O\,g^{-1}\,h^{-1}$) determined at 35 °C. However, variation between individuals was highest at extremes of T_a. There was a trend for increased conductance at 1 °C to be matched by the water loss, but this was not significant (Fig. 3A,B).

The total 'wet' thermal conductance (C_{TOTAL}) of *N. gouldi* was maximal at T_a=35 °C (24.25±1.99 $J\,g^{-1}\,h^{-1}\,°C^{-1}$) and minimal at T_a=10 °C (1.16±0.26 $J\,g^{-1}\,h^{-1}\,°C^{-1}$) (Fig. 3B). This decrease represented a 96 % decline in the rate of heat loss. Total thermal

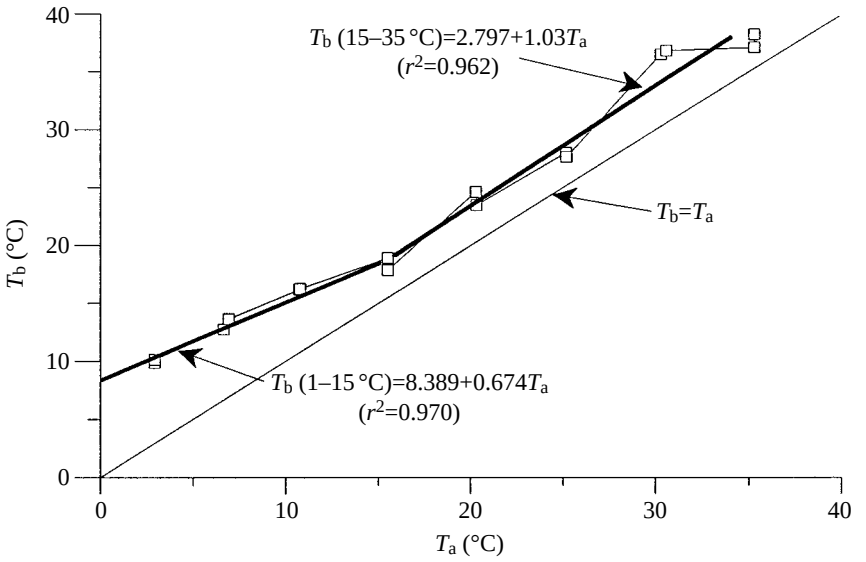

Fig. 2. Body temperature (T_b) plotted against respirometer chamber temperature (T_a). Individual symbols for T_b represent individual measurements, from a total of 16 bats exposed to the complete T_a range, with data from two bats randomly assigned to each temperature to permit analysis of variance. Between 1 and 15 ˚C and between 15 and 35 ˚C the slopes of the lines for T_b were significantly different from each other ($F_{1,14}$=9.584). For details see text.

conductance at 1 ˚C and 5 ˚C (3.38±0.65 J g^{-1} h^{-1} ˚C^{-1}) was significantly increased from the minimum value ($F_{4,10}$=10.22). Dry thermal conductance (C_{DRY}) exhibited the same trend as C_{TOTAL} with respect to decreasing T_a, but with some interesting differences due to the varying importance of evaporative heat loss (EHL). At T_a=35 ˚C, C_{DRY} was 14.25±1.99 J g^{-1} h^{-1} ˚C^{-1}, significantly lower than C_{TOTAL}. This difference remained significant as T_a decreased to 20 ˚C, when C_{DRY} was 2.42±0.34 J g^{-1} h^{-1} ˚C^{-1}. In accordance with the high TEWL, EHL was of considerable importance at high temperature (42±2.0 % of total conductance at T_a=35 ˚C), but contributed to a lesser extent at T_a=20 ˚C (26±0.4 %); at even lower T_a, the EHL was not a significant factor (Fig. 3B).

Breathing and gas exchange patterns

Rates of breathing and gas exchange varied over the T_a range 1–35 ˚C. At 1 and 5 ˚C, the pattern was irregular (i.e. variable but continuous), but between 10 and 25 ˚C intermittent (i.e. with apnoeic periods), while above 25 ˚C the bats' breathing became relatively constant and $\dot{V}_{O_2}$ more uniform (Fig. 4).

Ventilatory activity correlated closely with observed pattern of O_2 uptake. The longest period of apnoea/zero gas exchange measured during intermittent breathing was 9.15 min at 10 ˚C, but apnoea and a cessation of oxygen uptake also occurred at 15, 20 and 25 ˚C, although the duration of the periods decreased with increased temperature (Fig. 5A). Conversely, the frequency of gas exchange episodes increased with T_a from 0.38 min^{-1} at 10 ˚C to 0.85 min^{-1} at 25 ˚C (Fig. 5B). The volume of oxygen taken up per ventilatory/gas exchange episode showed no relationship to changes in T_a (Fig. 5C).

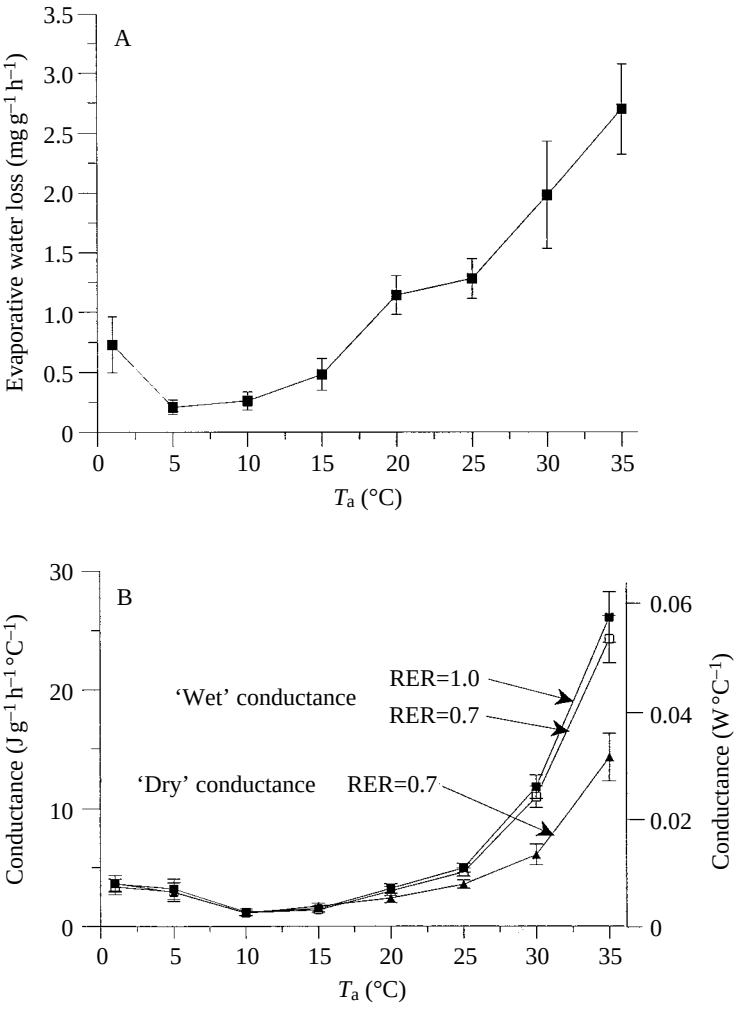

Fig. 3. The rates of water loss (A) and thermal conductance (B) exhibited by *Nyctophilus gouldi* between 1 and 35 °C (N=17). Thermal conductance is shown as both total 'wet' conductance (squares), calculated for two different values of RER of 1.0 (filled squares), and 0.7 (open squares), and 'dry' conductance (triangles, RER= 0.7), as described by Chappell and Roverud (1990). Values are mean ± S.E.M., N=5.

Gas exchange versus *temperature*

$\dot{V}_{O_2}$ varied with ambient temperature (T_a) in a curvilinear fashion (Fig. 6). The maximum $\dot{V}_{O_2}$ was 2.2±0.2 ml O_2 g^{-1} h^{-1} at 30 °C, which did not increase further at 35 °C. Below T_a=30 °C, $\dot{V}_{O_2}$ decreased to a minimum of 0.35±0.06 ml O_2 g^{-1} h^{-1} at 15 °C (84 % reduction over maximum rate). Below 15 °C, $\dot{V}_{O_2}$ increased with decreased T_a so that, at T_a=1 °C, $\dot{V}_{O_2}$ was not significantly different from the maximum $\dot{V}_{O_2}$ determined at an ambient temperature of 30 °C. Variation between individual bats was greatest at the temperature extremes (Fig. 6).

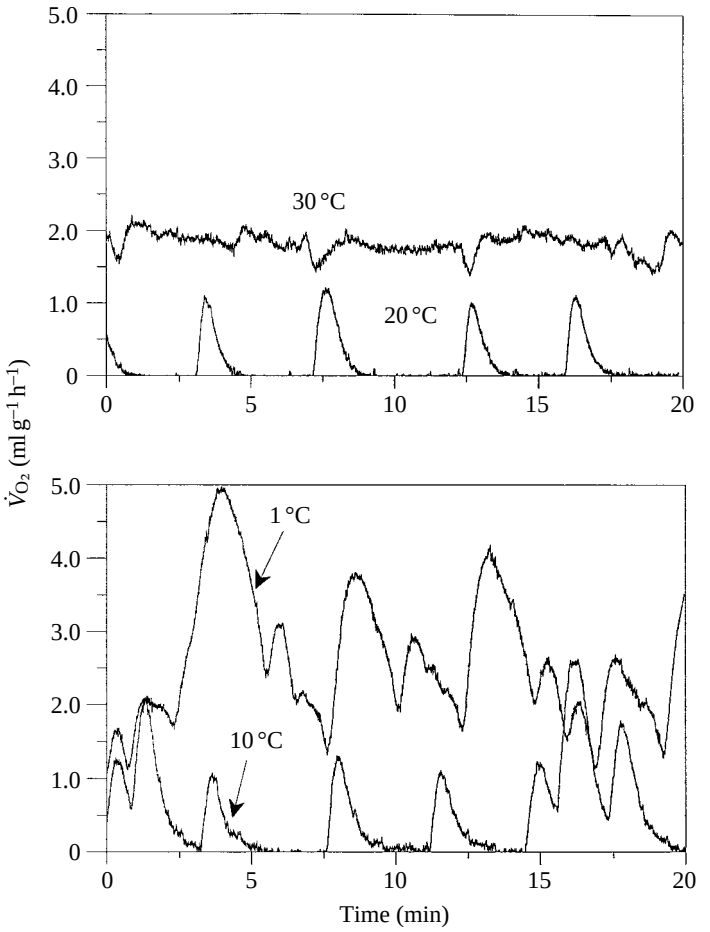

Fig. 4. Typical gas exchange patterns exhibited by an individual *Nyctophilus gouldi* over the experimental temperature range.

The relationship between $\dot{V}_{CO_2}$ and T_a was similar, except that the minimum $\dot{V}_{CO_2}$ occurred at 10 ˚C (0.17±0.04 ml CO_2 g^{-1} h^{-1}), which was an 87 % reduction from the maximum $\dot{V}_{CO_2}$. Again, it should be noted that the machine-originated error of these measurements is greatest at the lowest CO_2 production rates, although the trend is clear. While the minimum $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ occurred at different values of T_a values (15 ˚C and 10 ˚C respectively), which was reflected by a very low respiratory exchange ratio at 10 ˚C (RER=0.36±0.05), the gas exchange values at 10 ˚C and 15 ˚C were not significantly different.

The dichotomous response of *N. gouldi* to decreasing T_a from 35 to 1 ˚C was most apparent when plotted with respect to the temperature gradient, $T_b - T_a$ (Fig. 7). Decreasing T_a from 35 to 15 ˚C was accompanied by initially constant $\dot{V}_{O_2}$, despite an approximately 2 ˚C increase in $T_b - T_a$ (likely thermal neutral zone, TNZ), and then a marked decrease in $\dot{V}_{O_2}$ accompanying a further increase in $T_b - T_a$ of less than 1 ˚C

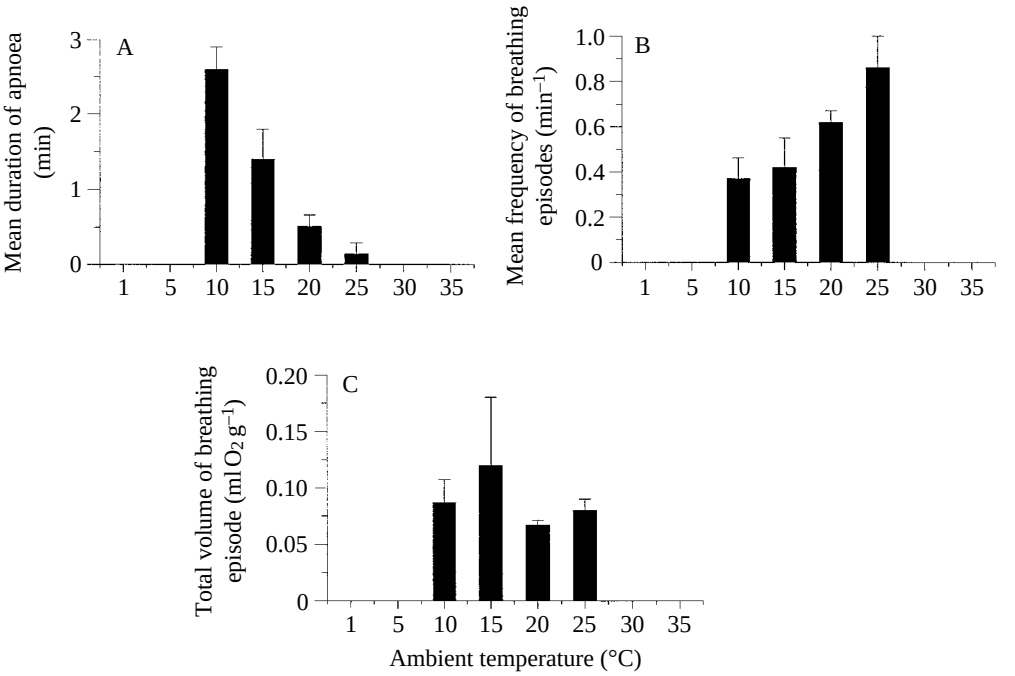

Fig. 5. The breathing/$\dot{V}_{O_2}$ variables of five *Nyctophilus gouldi* measured during intermittent respiration. (A) The duration of apnoeic periods; (B) the frequency of breathing episodes; and (C) the mean volume of oxygen taken up in each breathing episode. At 5 ˚C and below, as well as above 30 ˚C, breathing was continuous. Values are mean + S.E.M.

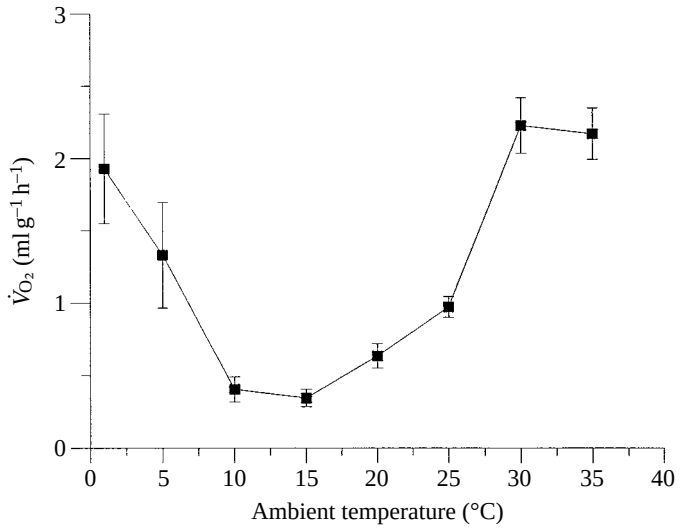

Fig. 6. The rate of oxygen uptake ($\dot{V}_{O_2}$) by *Nyctophilus gouldi* (*N*=17) at ambient temperatures between 1 and 35 ˚C, illustrating the likely thermal neutral zone (TNZ) (30–35 ˚C) and the torpor (30–15 ˚C) and low-temperature arousal (15–1 ˚C) ranges. Values are mean ± S.E.M.

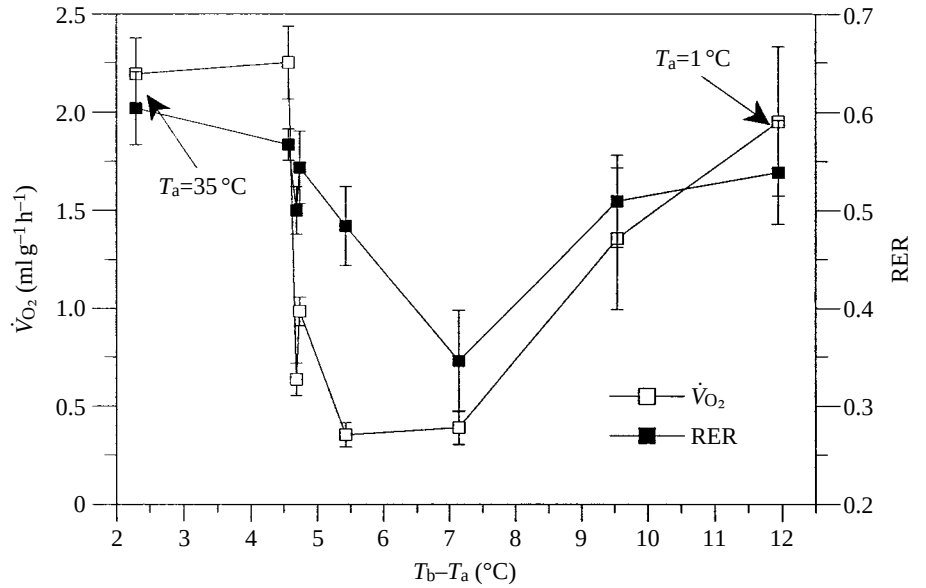

Fig. 7. *Nyctophils gouldi* $\dot{V}_{O_2}$ and respiratory exchange ratio (RER) with respect to the $T_b - T_a$ gradient. Values for all bats are shown, N=17, mean ± S.E.M.

(initiation of torpor). However, reducing T_a from 15 to 1 ˚C was accompanied by a larger increase in $T_b - T_a$ of almost 5 ˚C caused by a progressive increase in metabolic rate and thus endothermy (Fig. 7). Interestingly, while the RER exhibited a similar relationship with respect to $T_b - T_a$, the relative rate of decrease with lowering of T_a was slower than for $\dot{V}_{O_2}$. Thus, while $\dot{V}_{O_2}$ decreased promptly with a small change in the $T_b - T_a$ gradient, the relative $\dot{V}_{CO_2}$ decrease was greater.

Gas exchange during apnoea and torpor

Unusually low and high RER values were obtained from torpid bats. Variable and extreme RER values suggest that intermittent breathing and discontinuous gas exchange may be associated with CO_2 storage and pulsed excretion. Both CO_2 and O_2 exchange were intermittent and, although O_2 uptake was occasionally zero, the CO_2 excretion recordings frequently showed zero release (<0.01 %) for several minutes. Although not apparent from the long-term recordings (Fig. 8), the transition from minimal to peak gas exchange was not abrupt but a rather a ‘ramped’ increase. Fasted *N. gouldi* held at 35 ˚C exhibited relatively constant RER values with a mean of 0.77 (Table 1). The rates of CO_2 and O_2 exchange also remained relatively constant at this temperature, but decreased and became extremely variable when the bats were cooled to 15 ˚C and became torpid (Fig. 8). During several hours of torpor, RER ranged from 0 to greater than 1, occasionally nearly 2 (Fig. 8), while the mean RER was not significantly reduced (RER=0.64, Table 1; paired *t*-test). A return to 35 ˚C causing arousal was accompanied by a transiently elevated gas exchange and RER (Fig. 8) but no significant, sustained increase in RER during the return to continuous ventilation (Table 1). The RER values

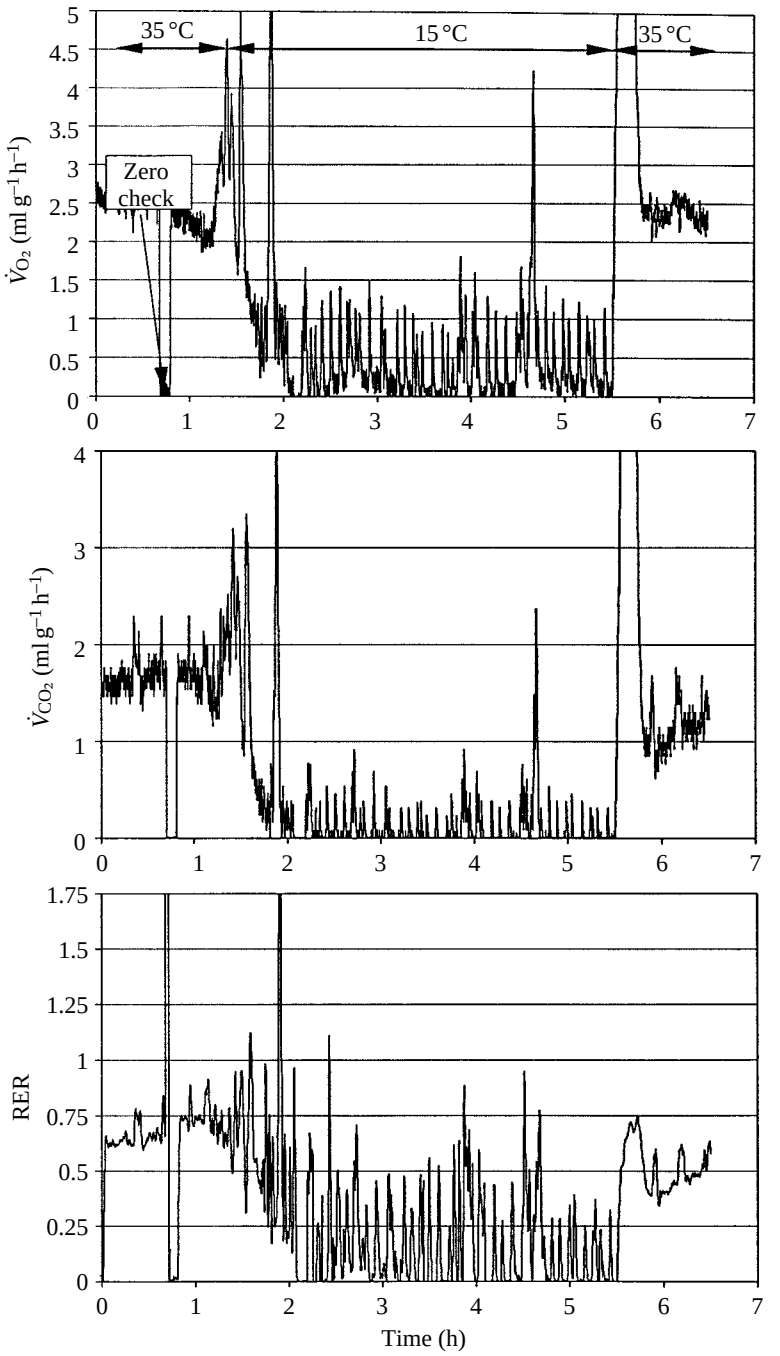

Fig. 8. Typical records for $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and calculated RER from *Nyctophilus gouldi*, in the awake continuously breathing state (35 ˚C), during torpor (15 ˚C) and during and after arousal (35 ˚C). Note the somewhat more 'quantized' nature of the $\dot{V}_{CO_2}$ records, compared with the $\dot{V}_{O_2}$ records, due to the digitised signal of the CD-3A analyser, and thus some reduction in resolution. Accordingly, small changes in RER should be interpreted with circumspection.

Table 1. *Changes in the respiratory exchange ratio (RER) of seven* Nyctophilus gouldi *at 35 °C before, during hypothermia-induced apnoea at 15 °C (torpor) and during and after arousal at 35 °C*

	Mean RER (35 °C) before arousal	Mean RER (15 °C) during torpor	Maximum RER during arousal	Mean RER during arousal	Mean RER (35 °C) after arousal
	0.79	0.60	1.05	0.63	0.60
	0.83	0.58	0.84	0.63	0.76
	0.76	0.74	0.80	0.65	0.68
	0.84	0.96	1.05	0.62	0.68
	0.61	0.61	0.94	0.75	0.64
	0.75	0.41	0.73	0.55	0.70
	0.79	0.58	0.87	0.63	0.70
Mean	0.77±0.08	0.64±0.17	0.90±0.12	0.64±0.06	0.68±0.05

determined here are sensitive both to failure to correct for time lag between O_2 and CO_2 detectors and to spurious transients in the records of either gas concentration. The variable RER values are not short-term transients but generally clearly require many minutes to increment gradually and then to decline.

Effect of feeding

Unfed bats rapidly became torpid after a brief settling period at 25 ˚C in the respirometer. Fed bats showed, however, an elevated $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and body temperature (T_{sur}) indicative of generally higher metabolic rates and sustained homeothermy (Fig. 9A,B,C). Fed bats exhibited a peak $\dot{V}_{O_2}$ of 5.6 ml g^{-1} h^{-1}, 500 % of the rate of torpid *N. gouldi*. Interestingly, the difference in $\dot{V}_{CO_2}$ was even larger, showing a relative increase of more than 850 % of the torpid rate. After settling, there was no significant change in $\dot{V}_{O_2}$, $\dot{V}_{O_2}$ and T_{sur} for unfed bats, but a highly significant decrease in all three metabolic indices in the fed animals ($F_{2,6}$=255, 644, 10.21, respectively). The disproportionate increase in $\dot{V}_{CO_2}$ compared with $\dot{V}_{O_2}$ in the fed animals is apparent in the significantly greater RER values ($F_{1,2}$=28.7) of fed bats (RER≈1) (Fig. 10). Although there was no real correlation between RER and body temperature in fed bats, both returned to the torpid rates after 9 h (Figs 9, 10). Unfortunately, it was not possible to induce fasted bats to maintain a high T_b at T_a=25 ˚C without including an extra activity component and therefore it was difficult to ascertain what fraction of the relatively elevated metabolic rates was due to the ‘specific dynamic action’ of feeding.

Discussion

Clearly *N. gouldi* did not exhibit the normal homeothermic respiratory pattern, but instead showed that an extremely labile metabolic rate and standard (or basal) metabolic rate must occur above 30 ˚C in fasted bats. Environmental and physiological variables, such as temperature and nutritional status, have a marked effect on the metabolic rate of *N. gouldi*.

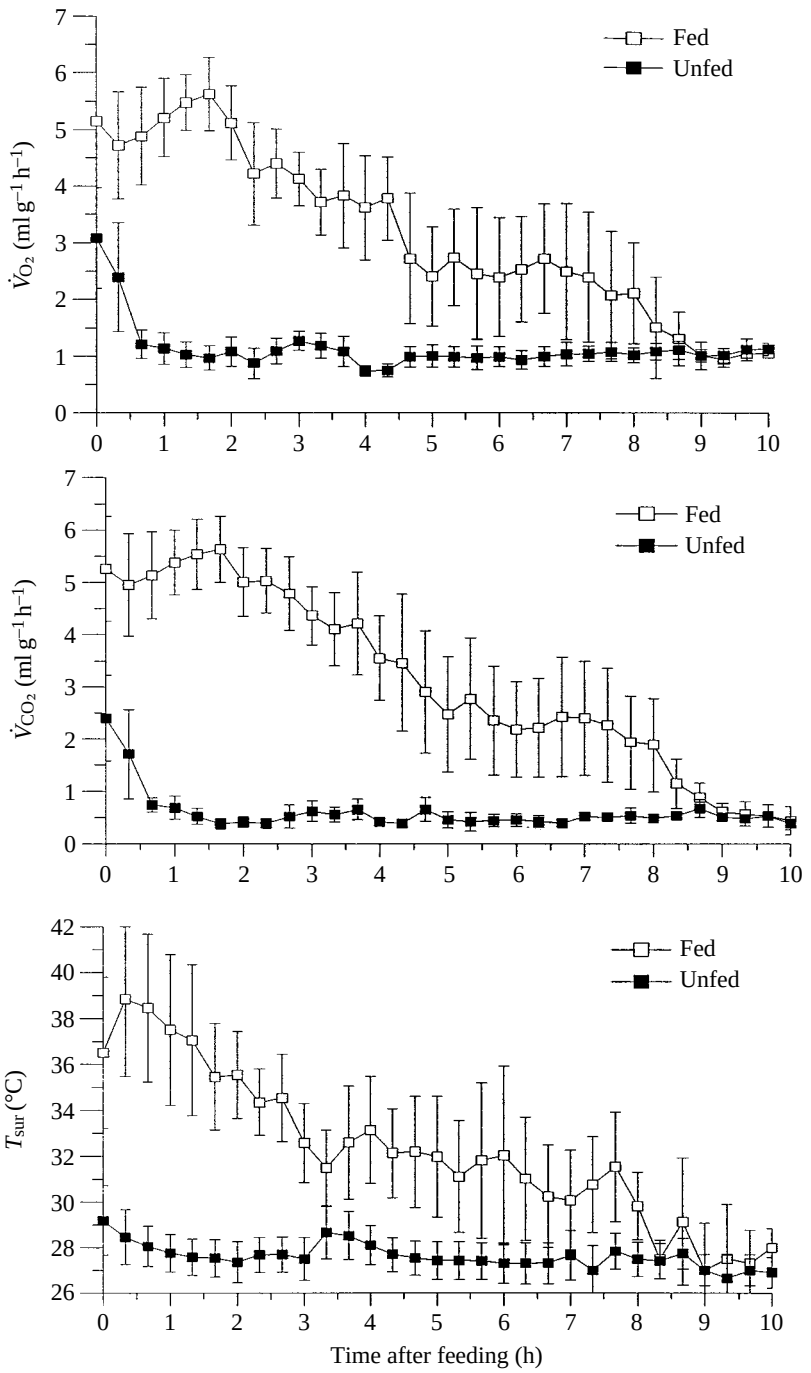

Fig. 9. The rates of oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide excretion ($\dot{V}_{CO_2}$) and the surface body temperature (T_{sur}) for fed and unfed *Nyctophilus gouldi* (N=12). Values are mean ± S.E.M.

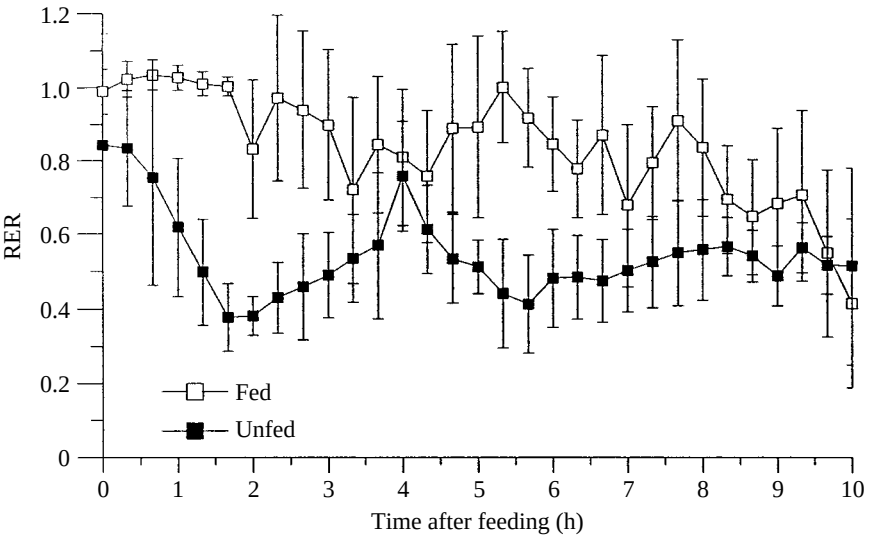

Fig. 10. The calculated RER of the fed and non-fed bats shown in Fig. 9 (N=12). Values are mean ± S.E.M., N=12.

Gut passage and retention time

N. gouldi are post-absorptive 9 h after feeding, which contrasts with a mean retention time (MRT) suggesting that the digestive period was approximately 5 h. Thus, MRT is probably a misleading index of the post-absorptive state in as much as the metabolic effects of feeding were evident for much longer. The t_{100} (i.e. the time until 100 % of the marker is defecated) is more appropriate since, while any food remains in the gut, some absorption is possible. In this study, t_{95} (9 h) corresponds with the duration of the metabolic effects of feeding. The large time lag between t_{95} and t_{100} (16 h) suggests that some digesta may have been stored in the rectum, where little reabsorption is likely to occur (Warner, 1981). Studies of gut transit time in the microbats *Eptesicus fuscus* and *Myotis lucifugus* also suggest rectal storage of digesta, which is excreted at the onset of the next foraging session (Luckens *et al.* 1972; Buchler, 1975).

The MRT of *N. gouldi* is more than double that of other small insectivorous mammals (Table 2). Comparisons between studies are complicated by the absence of standard marker methods and the combined difference in reproductive condition, age, meal size, activity levels and ambient temperature. For example t_0 for *N. gouldi* in the present investigation (1.3 h) is greater than that reported by Grant (1988) for the same species. However, Grant (1988) exercised his *N. gouldi* after feeding, whereas in the present study the bats remained in the enclosures. Buchler (1975) also found that post-feeding activity affected t_0. The bats were kept inactive in the present study so that MRT could be compared with metabolic events associated with feeding. As to the relatively long retention time of *N. gouldi*, there are several considerations. First, pollen may have been an inappropriate marker and may have caused retention time to be overestimated, but the close correspondence between gut clearance and the decline in metabolic rate suggests that the pollen method was quite exact. Second, the high digestible energy content

Table 2. *Retention times (h) in the gut of bats and selected Insectivora*

Species	Procedure (marker)	t_0	MRT	t_{95}	t_{100}	Reference
Nyctophilus gouldi	Particulate	1.3	5.4	9	25	This study
		1				Grant (1988)
Insectivora						
Least shrew	Arthropods	0.4			1.8	Skaren (1978)
Water shrew	Mealworms		1.3		4.3	Kostelecka-Myrcha and
	Wheat in mealworms		2.3		10	Myrcha (1964, 1965)
Megachiroptera						
Syconycteris australis	Pollen	0.7	2.4		9	Law (1991)
Pteropus vampyrus	Dust and banana	0.5	2	4.5	5.5	Richardson *et al.* (1987)
Cynopteris brachyotis	Sephadex G25 and banana	0.5	4.5	10	12	Richardson *et al.* (1987)

MRT, mean retention time; t_0, time of first defecation; t_{95}, t_{100}, times at which defecation is 95 % and 100 % complete.

(lipoprotein) of mealworms (Wigglesworth, 1961) may account for the differing MRT of *N. gouldi* compared with that of flying foxes (fed fruit) (Richardson *et al.* 1987; Table 2), but could not account for similar differences compared with other insectivorous mammals. Third, bats exhibiting low mass-specific metabolic rates may require longer to process their food. In comparison with other insectivores, such as shrews, *N. gouldi* has both a lower mass-specific metabolic rate (McNab, 1983) and a longer MRT (Warner, 1981). Investigations of gut morphology and the relationship between digestion and metabolism may be useful in explaining such differences.

Thermobiology and torpor

While homeotherms respond to temperatures above the thermal neutral zone by increasing their metabolic rate, the increase in metabolic rate for *N. gouldi* when T_a increased from 15 to 30 ˚C represents an arousal from torpor. The rate of water loss and thermal conductance were also greatest at high temperature, increasing significantly between 30 and 35 ˚C despite evidence of stabilising T_b (approximately 37.5 ˚C for *N. gouldi*) and a clear plateau in $\dot{V}_{O_2}$ (cf. Figs 2, 6 and 7). Stable metabolic rate and T_b, while T_a, conductance and water loss rise, are indicative of the TNZ and are part of the normal thermoregulatory response of homeotherms in preventing T_b rising to lethal levels (Schmidt-Nielsen, 1990).

Below 30 ˚C, instead of maintaining a constant body temperature by increasing metabolic rate, as is the homeothermic pattern, fasted *N. gouldi* exhibited heterothermy and became torpid. Reduced metabolic rate and T_b occurred down to 10 ˚C. Notably, at T_a values below 10 ˚C, *N. gouldi* significantly reduced the rate of decrease in body temperature while metabolic rate increased rapidly in an apparent attempt to stabilise T_b. Therefore, while *N. gouldi* can obviously tolerate and survive T_a values below 0 ˚C, the bats increasingly elevate metabolic rate when T_b declines below 20 ˚C (when $T_a \leqslant 10$ ˚C).

Thermal conductance in homeotherms is typically constant below the thermal neutral zone (TNZ) (Aschoff, 1981). In *N. gouldi*, there was compelling evidence that the TNZ of fasted bats was above a T_a of 30 ˚C when T_b reached approximately 37.5 ˚C. Several

Table 3. *Total 'wet' and 'dry' thermal conductance values, showing the advantages of torpor for water conservation in* Nyctophilus gouldi

Species	T_a (°C)	C_{TOTAL}	C_{DRY}	Condition	Source
Plecotus auritus	25	28.8		Euthermic	Webb *et al.* (1993)
(mass≈12.5 g)	5	63.4			
Noctilio albiventris	35		6.0	Euthermic	Chappell and Roverud (1990)
(mass≈40 g)	10		3.7		
Nyctophilus gouldi	35	24.3	14.2	Aroused, TNZ	This study
(mass≈8 g)	5	3.4	3.3	Torpid	

Conductance (C) has been converted to J g^{-1} h^{-1} °C^{-1}.
TNZ, thermal neutral zone.

species of subtropical tree bats, genus *Lasiurus* and *Nycticeius numeralis*, have TNZs above 31 ˚C (Genoud, 1993). Below this TNZ, torpid bats exhibited a generally lower, but variable, thermal conductance down to 1 ˚C. Changes in the posture, skin perfusion, T_b and metabolic rate of the bats can all cause thermal conductance to vary with temperature. The thermal conductance of *N. gouldi* was between 1.5- and fourfold greater than that of a 10 g homeothermic organism, which is typical for bats because of their large surface area to volume ratio (Bradley and Deavers, 1980; see Introduction). While the thermal conductance of *N. gouldi* also appears to be much greater than those of other torpid bats (e.g. *Myotis lucifugus*; Henshaw, 1968), comparison with available conductance values for bats shows that *N. gouldi* is unexceptional (Table 3). However, *N. gouldi* has a marked ability to lower conductance, and thereby to conserve energy and water, at relatively high T_a (Table 3). High conductance would require relatively greater energy utilization if *N. gouldi* were to maintain a body temperature similar to those of other bats. Indeed, comparisons with a number of other species of small insectivorous bat, with a propensity for torpor, indicate that metabolic rates can be very variable between species and that torpid *N. gouldi* have one of the highest rates of oxygen consumption (Table 4). Relatively high thermal conductance and metabolic rates and, therefore, on a mass-specific basis, greater food requirement may increase the frequency of torpor in *N. gouldi* (compared with other bats) and may explain why the species becomes torpid at temperatures as high as 25 ˚C.

Torpor typically reduces evaporative water loss in birds and mammals (Lasiewski, 1964; Carpenter, 1969; Bell *et al.* 1986). Torpid and homeothermic humming birds showed a three- to sixfold difference in evaporative water loss (Lasiewski, 1964), while the bat *Eptesicus fuscus* shows a two- to fourfold difference (Carpenter, 1969). The reduction in water loss by torpid compared with fully aroused *N. gouldi*, within the TNZ (T_a=35 ˚C) was even more marked, showing a four to tenfold decrease.

The measured rates of evaporative water loss in *N. gouldi* are markedly lower than those for the temperate cave-dwelling bat *E. fuscus* (Carpenter, 1969) and the tropical cave-dwelling species *Macroderma gigas* and *Rhinonicteris aurantius* (R. Baudinette, personal communication). This difference can be correlated with the different roosting

Table 4. *Oxygen uptake rates for heterothermic microchiroptera at low ambient temperature*

Species	Temperature (°C)	Condition	Oxygen uptake ($ml\,g^{-1}\,h^{-1}$)	Source
Eptesicus fuscus	10	Torpid	0.034	Szewczak and Jackson (1992*b*)
Myotis lucifugus	5	Torpid	0.02	Thomas *et al.* (1990)
Myotis velifer	5	Torpid	0.07	Riedesel and Williams (1976)
	10	Torpid	0.16	Riedesel and Williams (1976)
Pipistrellus pipistrellus	4–10	Torpid	0.232	Hays *et al.* (1991)
	4	Torpid	0.155	Speakman and Racey (1989)
Plecotus auritus	10	Apnoeic	1.26	Webb *et al.* (1992)
	10	Continuous breathing	>9	Webb *et al.* (1992)
Noctilio albiventris	10	Non-torpid	5.28	Chappell and Roverud (1990)
	30	Non-torpid	0.99	Chappell and Roverud (1990)
Nyctophilus gouldi	10	Torpid	0.38	This study
	30	Non-torpid	2.26	This study

conditions of the two bats, since it would be advantageous for tree-dwelling species, such as *N. gouldi*, to minimise evaporative water loss to the environment because relative humidity in a tree roost would vary more and often be considerably lower than in caves. Field water turnover estimates would be useful in determining to what extent water is limiting in *N. gouldi* and microbats generally. At low temperatures, metabolic rate is reduced in torpid *N. gouldi*, conserving energy stores. Reduced $\dot{V}_{O_2}$ can be facilitated by a reduction in ventilation rate and/or tidal volume, significantly reducing evaporative water loss. Speakman and Racey (1989) also concluded that dehydration was potentially crucial and that *P. pipistrellus* emerge in winter to drink rather than to feed, while Hays *et al.* (1992) concluded that energy and water replenishment were likely to be of equal importance in stimulating winter flying of *P. auritus*. Hibernating little brown bats, *M. lucifugus*, also seem to show an arousal and torpor pattern dependent on TEWL (Thomas and Cloutier, 1992), but they extended the duration of torpor by reducing pulmonary EWL to less than 1 % of TEWL. Thus, low levels of water loss during torpor may serve to extend the duration of dormancy by reducing the drinking requirements of the bats. *Noctilio albiventris* makes a corresponding saving in EHL, which is reduced from 36 to 2.7 % of total heat loss when T_a decreases from 35 to 1 °C (Chappell and Roverud, 1990). Below 10 °C, *N. gouldi* attempts to defend body temperature against further decreases by large increases in metabolic rate (increased $\dot{V}_{O_2}$), accompanied by an escalation of the rate of water loss and conversion of mass into metabolic water, but there is no apparent effect on the contribution of EHL. The EHL falls from 42 % (T_a=35 °C) of total conductance to near zero at T_a=15 °C and below. In fact, the TEWL of $0.21\,mg\,g^{-1}\,h^{-1}$ for *N. gouldi* (T_a=1–5 °C) is low compared with approximately $0.8\,mg\,g^{-1}\,h^{-1}$ calculated for *Myotis* (T_a=4 °C) (Thomas and Cloutier, 1992).

The natural clustering behaviour of roosting *N. gouldi* (Richards, 1983) would reduce water and heat loss in the wild. Clustering may increase roost temperature by 2–3 °C (Davis, 1970), and the reduction in surface area has been shown to be effective for the

bats *Myotis lucifugus* (Kurta *et al.* 1987), *Phyllostomus discolor* (McNab, 1969) and *Noctilio albiventris* (Roverud and Chappell, 1991) and for other small clustering animals, such as the naked mole rat (*Heterocephalus glaber*; Yahav and Buffenstein, 1991) and voles (*Microtus agrestis*; Hayes *et al.* 1992). Clearly this is not a long-term strategy, however, but rather potentially extends torpor by increasing the time to lethal dehydration and/or exhaustion of energy reserves. *N. gouldi* has a wide latitudinal distribution from near equatorial to temperate. It is likely that susceptibility to evaporative water loss, as well as to low temperature, is important in determining the distribution and success of chiropteran species.

Episodic respiration/gas exchange

Long-term reductions in gas exchange enabling periods of low ventilatory rate or apnoea in torpid *N. gouldi* (10–25 °C) will reduce evaporative water loss in expired air. This strategy has been demonstrated in a number of animals, most elegantly for the 'burst–flutter' pattern of the silkworm pupa (Levy and Schneiderman, 1966). Thus, intermittent breathing may not be simply dependent on optimising energy turnover. Intermittent breathing in mammals has been suggested as an economic strategy to reduce ventilatory costs and to permit a relatively reduced metabolic rate (Milsom, 1991). This behaviour has been observed in several species of bat during torpor (e.g. Thomas *et al.* 1990; Hays *et al.* 1991; Webb *et al.* 1992). Intermittent breathing by brown long-eared bats has been suggested by Webb *et al.* (1992) to be the optimisation of breathing frequency reducing ventilatory costs. However, the duration of apnoea in *N. gouldi* increased with decreased $\dot{V}_{O_2}$ down to 15 °C, while between 10 and 15 °C significant increases in the length of apnoea were not accompanied by a decreased metabolic rate. Similarly, in the bat *Pipistrellus pipistrellus*, intermittent breathing did not correspond directly with metabolic rate in the minimum zone (4–10 °C), where metabolic rate is independent of temperature (Hays *et al.* 1991). Similar conclusions by Hays *et al.* (1991) about the dependency of intermittent breathing and $\dot{V}_{O_2}$ in other bats may, however, be premature for species other than the pipistrelle. Other studies have also suggested resumption of regular breathing and gas exchange at low temperature (e.g. hedgehogs, Sovio *et al.* 1968; ground squirrels, Hammel *et al.* 1968).

The link between the duration of apnoea and temperature is especially interesting. While the quantity of oxygen consumed per episode did not change as temperature decreased, the duration of apnoea increased significantly. The stimulus for breathing in mammals is thought to be the CO_2/HCO_3^- content of the hypothalamic fluid, providing for a CO_2-driven respiratory centre (Malan, 1985). The volume of oxygen taken up by *N. gouldi* in each breathing episode was consistently the same, presumably leading to the production of a consistent quantity of CO_2 (RER=1–0.7). Are the bats inhibiting the stimulus to breath, in order to extend apnoea, as temperature decreases? If so, the 'set point' of the hypothalamus (alpha-stat) must be reset with each T_a, allowing more CO_2 to be stored in the body (alpha-stat theory; Reeves, 1972) and hence allowing extended apnoeic periods. Many hibernating mammals show a relative acidosis as a result of this 'resetting' (Bickler, 1984; Malan, 1985), and there is indication that episodic breathing by torpid bats is correlated with $P_{a_{CO_2}}$ (Szewczak and Jackson, 1992*b*). Unusually low and

transitional RER values determined at low temperature for *N. gouldi* are characteristic of increased CO_2 storage and low metabolic rates, permitting longer lengths of apnoea in *N. gouldi*. While some adjustment of CO_2 storage occurs with changed T_b, it seems to be a more important consequence of breath-holding. There was no significant difference in mean RER values in aroused/continuously breathing, torpid and post-torpid *N. gouldi*, implying similar, steady-state gas exchange. However, high RER was determined in association with each breathing episode in torpid bats, often near the start of each episode. One implication is that 'excess' CO_2 stored during the preceding apnoea is flushed during the initial phase of the ventilatory/exchange spell, but with normal gas exchange rates occurring in the remaining period of the episode. Wide fluctuations in RER associated with breath-holding are common in diving vertebrates (Seymour, 1989). Therefore, it seems imperative that further investigations attempt to discriminate between CO_2 accumulation as a result of breath-holding and increased 'background' CO_2 storage promoting a relative acidosis during torpor at low temperature.

There is some debate as to the relative importance of episodic breathing, since available data are unable to resolve whether $\dot{V}_{O_2}$ during the episodes is sufficient to account for the total $\dot{V}_{O_2}$ over longer periods of torpor. Hays *et al.* (1991) suggest that the glottis remains open during apnoea in *P. pipistrellus* and that diffusive O_2 uptake occurs with some diffusive CO_2 loss, supplementing gas exchange during breathing. Conversely, Thomas and Cloutier (1992), working with *M. lucifugus*, calculated that $\dot{V}_{O_2}$ during breathing episodes is sufficient to account for all oxygen uptake and that the glottis remains closed during apnoea. It might be possible to correlate these dichotomous observations with the high and very low metabolic rates of *Pipistrellus* and *Myotis* respectively (Table 4). However, the present study of *N. gouldi* revealed that there was little oxygen uptake between breathing episodes and that the integrated O_2 uptake of several breathing episodes ($0.48\,ml\,h^{-1}\,g^{-1}$) compared with the relatively high mean $\dot{V}_{O_2}$ ($0.48\,ml\,h^{-1}\,g^{-1}$) of torpid bats was sufficient to account for all $\dot{V}_{O_2}$.

The pattern of intermittent breathing and gas exchange, designed in part to conserve water, is highly reminiscent of the water-conserving 'burst–flutter' pattern of insect pupal respiration (Levy and Schneiderman, 1966). Importantly, breath-holding and CO_2 storage in the tissues and body fluids results in increasing negative pressure in the respiratory system when the spiracles are closed; slight opening of the spiracles permits air entry with no water or CO_2 loss (the flutter). Eventually, CO_2 levels increase to the point where a breathing episode is required (the burst). It is interesting to speculate on the case of apnoeic bats. While CO_2 storage undoubtedly occurs in diving vertebrates, the conclusion is less certain for torpid mammals. Malan *et al.* (1973) determined that arterial pH in apnoeic, hypometabolic marmots at lowered T_b never deviated by more than 0.045 units, but also point out that slowed metabolism and perfusion would require several minutes for the acid accumulation and increased P_{CO_2}. Szewczak and Jackson (1992*a*) provide compelling evidence of both acidotic end-ventilatory blood pH and important cyclic changes in blood pH of apnoeic, torpid bats, *Eptesicus fuscus*. This bat shows clear evidence of CO_2 accumulation during breath-holding with a mean cycle from 28 to 46 mmHg P_{CO_2}. If CO_2 storage occurs during apnoea, then clearly, with a closed air-way, lung pressure could become negative, relative to that of air, providing a mechanism for

mass air influx. Thus, although the glottis may normally be closed, possibly resulting in negative lung pressure, only small movements of the glottis would be required to permit entry of air, supplementing O_2 uptake during breathing without loss of water or CO_2. The retention and storage of CO_2, depressing metabolism, does not necessarily require a closed glottis (Thomas *et al.* 1990). Such behaviour may be species-dependent and it has been implicated by data for torpid *Eptesicus fucus* (Szewczak and Jackson, 1992*a*,*b*) or it may vary with circumstance and temperature. However, this role for the glottis requires increased CO_2 storage and transitional high RER values on initiation of the next breathing bout. Precise and extensive determinations of RER in euthermic and torpid bats from several species are required to assess this proposal. In the absence of verifiable CO_2 storage, the contentions of Hays *et al.* (1991) should gain ascendance. Only a few of the contemporary studies of respiration in bats have determined $\dot{V}_{CO_2}$ and thus RER (e.g. Chappell and Roverud, 1990), but the long-term average RER values for fasted *N. gouldi* in the present study are somewhat less than 0.7, the value normally assumed for fat metabolism. The exact value of RER is dependent on the C:H:O ratios of the fat, and the low values for bats may indicate preferential use of fats, producing large amounts of metabolic water.

Decreasing ambient temperature down to 10 °C caused *N. gouldi* to increase the duration of apnoea in response to decreased oxygen demand, but without changing the amount of gas exchange occurring in each episode. In torpid *Eptesicus fuscus*, both hypoxia and hypercapnia stimulated ventilation, leading to the conclusion that *Eptesicus* over-ventilates with respect to its O_2 requirements (Szewczak and Jackson, 1992*a*,*b*). Considering both the data from the literature and the data for *N. gouldi*, it is likely that the duration of ventilatory episodes is determined by the return of P_{CO_2} to a set point and that a constant overall RER will relate to a fixed $\dot{V}_{O_2}$. Since the relationship between P_{CO_2} and acid–base variables is temperature-dependent, there must be some adjustment of the 'set point'. Hence, the role of CO_2 in determining both the pattern and the duration of ventilation in torpid bats requires considerable further investigation.

Feeding and torpor

The effect of feeding on the metabolic rate of *N. gouldi* was to obviate torpor, since body temperature and gas exchange were typical of aroused and continuously breathing bats. The effect of feeding and digestion was to elevate the metabolic rate of *N. gouldi* for 9 h post-feeding, after which the bats became torpid. The peak $\dot{V}_{O_2}$ produced by digestion in *N. gouldi* was similar in magnitude to that measured in poikilotherms such as fish (Jobling, 1981; Carey *et al.* 1984) and reptiles (Lang, 1979; Lysenko and Gillis, 1980).

It seems to be of major importance that it was only in the immediately post-feeding phase that RER approached 1.0, a value typical of carbohydrate metabolism. After 2 h of digestion, RER began to decline, although it is difficult to assess whether this was due to the concomitant decline in body temperature, which promoted an increased CO_2 storage, or to the rapid exhaustion of carbohydrates from the meal. It is clear, however, that within a few hours of feeding the animals became torpid and that RER declined to values indicative of fat utilisation.

The ability of *N. gouldi* to move directly from a homeothermic metabolic state to a

'poikilothermic' state at the completion of digestion is probably advantageous in conserving energy that would otherwise be utilised to maintain homeothermic body temperature. Provision of more food may have inhibited torpor in this species, since in the related *N. geoffreyi* an experimentally temperature-dependent food supply induced torpor more frequently than an *ad libitum* food supply (Ellis *et al.* 1991). It is possible that the captive conditions, or the use of small respiratory chambers, may have induced torpor in *N. gouldi*, a condition known as 'confinement hypothermia' (Henshaw, 1970). Alternatively, the adoption of torpor by *N. gouldi* may have been the natural seasonal response, as low metabolic rates, and possibly even hibernation, in *N. gouldi* are required during winter to prevent early fertilisation and the development of young when food is scarce (Phillips and Inwards, 1985).

The results of a previous study of *N. gouldi* (Phillips, 1984), in which torpor could only be induced through starvation, contrast with those of this study, presenting an interesting dilemma. Was torpor, as apparent in this investigation, a natural consequence of season or was it an artefact of the experimental conditions? Further investigation is required to answer this question; however, it is most significant that *N. gouldi* are able to become torpid over a wide range of temperatures and are thus able to conserve energy when required.

N. gouldi exhibit a highly labile metabolic rate which may be directly influenced by temperature and nutritional status. Having also found that *N. gouldi* are relatively efficient water conservers, it would be interesting to determine the effect of relative humidity on the metabolism of this species in comparison with that of other tree- and cave-dwelling bats and also to study the effect of long-term food availability on metabolic rate.

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